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Ecophysiological Differences Between Sterile and Fertile Fronds of the Subtropical Epiphytic Fern *Pyrrosia lingua* (Polypodiaceae) in Taiwan

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Abstract.—Many ferns have specialized fronds that bear sporangia, whereas sterile fronds lack reproductive structures. Although a strong case can be made that the presence of the sporangia will affect the physiology of the frond, only one study could be located that investigated this phenomenon. Thus, ecophysiological (and some morphological) features of fertile fronds were compared with those of sterile fronds of the subtropical epiphytic fern *Pyrrosia lingua* in Taiwan. Fertile fronds were thicker than sterile fronds, a result of the presence of the large sori. Stomatal sizes and densities did not differ between the two types of fronds. The osmotic potential of liquid expressed from the fertile fronds was more negative than that of the liquid of sterile fronds, although this may be an artifact due to a matric effect of the released spores. No differences in chlorophyll concentrations (area basis only) and *a/b* ratios were found between sterile and fertile fronds. *In situ* rates of net CO_2 exchange of the fertile fronds were substantially lower than those of the sterile fronds. Similar stomatal conductances and internal CO_2 concentrations in the sterile fronds indicated that the efficiency of the photosynthetic apparatus was lower in fertile relative to sterile fronds. The results of this study indicate that the presence of sori on fronds of the epiphytic fern *Pyrrosia lingua* reduces the photosynthetic capacity of these fronds and, most likely, the productivity of plants harboring many fertile fronds.

Ferns are unique among higher plants in that their foliar structures (fronds) carry the reproductive units of the plant (spores). Spores are borne in sporangia directly on the frond surfaces in sori (Raven *et al.*, 1999). In some cases, an entire surface of the frond, typically the abaxial one, is covered by sori (Wagner

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and Wagner, 1977; Hovenkamp, 1986). In some species of ferns, fronds bearing spores, fertile fronds, may co-occur with fronds lacking sporangia, sterile fronds. The dual role of such foliar structures, i.e., photosynthesis and reproduction, raises an interesting question regarding the potential trade-offs involved in the maintenance of these two functions. This question addresses how the presence of spores, especially on fronds with one surface almost completely obscured by sori, might affect photosynthesis in fertile, relative to sterile, fronds. Fronds covered with sori might exhibit lower photosynthetic rates as a result of decreased light interception by the abaxial surface, or the presence of sori on the surface of the frond might physically impede gas exchange. On the other hand, it is feasible that fertile fronds might exhibit higher photosynthetic rates as a result of an increased sink demand to supply the developing sporangia with carbohydrates.

Results from other source-sink studies comparing leaf photosynthesis on branches or in trees with and without fruits are mixed. In apple trees, photosynthetic rates of leaves on branches bearing fruit were higher than rates of leaves on branches or trees lacking fruit (Fujii and Kennedy, 1985; Faust, 1989). In contrast, photosynthetic rates of leaves on branches bearing reproductive structures did not differ from vegetative shoots in olive and in pine (Dick *et al.*, 1991; Proietti, 2000). Furthermore, in dioecious species in which female trees presumably experience greater carbohydrate demands, leaves on female trees exhibited higher photosynthetic rates or greater photosynthetic light-use efficiencies than leaves on male trees in boxelder and holly (Dawson and Ehleringer, 1993; Obeso and Retuerto, 2002), but not in pistachio (Correia and Diaz Borradas, 2000). In contrast to the last study, Vemmos (1994) found higher photosynthetic rates in fruit-bearing trees of pistachio early in the growing season, but no differences between trees with and without fruit in the second half of the growing season. Thus, it is difficult to generalize whether or not increased sink strength due to the presence of reproductive structures results in higher photosynthetic rates in nearby leaves.

Questions of the potential effect of reproduction on the physiological activity of nearby photosynthetic organs might best be addressed in fern taxa having fertile and sterile fronds, given that the reproductive structures (sori with sporangia) are located directly on the surface of the photosynthetic organ. Despite this, only one previous study could be located in which this question was addressed. Bauer *et al.* (1991) measured photosynthetic rates of sterile and fertile fronds of *Dryopteris filix-mas*, a terrestrial fern widely distributed in northern temperate regions, throughout the growing season. In this fern, sterile fronds are produced in two flushes, one in the spring and another in early summer. Fertile fronds are produced in the spring. Photosynthetic rates of all fronds varied greatly throughout the season, and differences between sterile and fertile fronds were not large. If comparison of the fertile and sterile fronds is limited to those fronds produced in the same flush, the net CO_2 uptake rates of the fertile fronds nearly always exceeded those of the sterile fronds. On the other hand, the photosynthetic rates of the sterile fronds produced later in the year were typically higher than those of the fertile fronds. Furthermore,

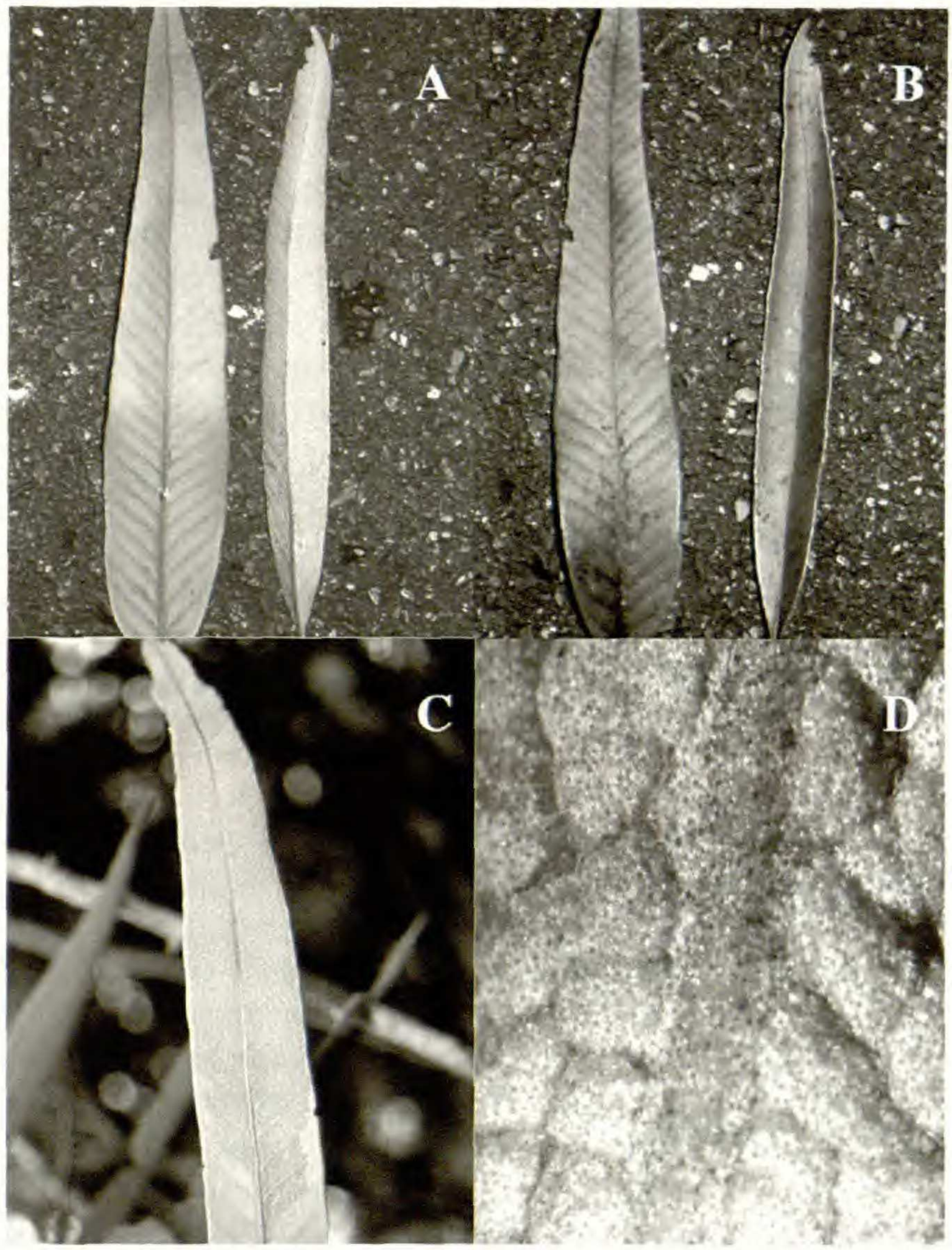

FIG. 1. Abaxial (A) and adaxial (B) surfaces of sterile (on the left) and fertile (on the right) fronds of *Pyrrosia lingua* in northeastern Taiwan. The abaxial surface of a frond with a partial covering of sori is depicted in C, and a close-up of the sori on the abaxial surface of a frond is shown in D. The fronds are approximately 20 cm long (A–C), and the sori are approximately 1 mm in diameter (D).

photosynthetic rates of the spring-flushing sterile and fertile fronds were similar at the beginning of a second year of measurements. Thus, based on the results of this study of the terrestrial fern *D. filix-mas* (Bauer *et al.*, 1991), it is difficult to generalize about the potential physiological costs of reproduction in the fronds of ferns, although it is clear that spore production in this fern did not dramatically reduce the photosynthetic rates of the fertile fronds.

An ideal candidate for further investigation of the physiological consequences of spore production in fern fronds is the subtropical epiphyte *Pyrrosia lingua*. This fern produces sterile and fertile fronds, and sori cover a variable percentage of the abaxial surfaces, with some fronds showing nearly complete coverage of the abaxial surface by sori (Fig. 1). At any point in time, numerous

sterile and fertile fronds can be found on this epiphyte, which grows densely along the trunks of many host tree species of subtropical rain forests. This fern seems to prefer more exposed locations of the tree canopy (personal observation).

The goal of this study is to determine the potential ecophysiological effects of the presence of sori on the fronds of an epiphytic, heterophyllous fern. Specifically, the aim was to compare ecophysiological (and some morphological) features of sterile and fertile fronds in the epiphytic fern *Pyrrosia lingua* in a subtropical rain forest in northeastern Taiwan. An emphasis was placed on features that relate to photosynthetic capacity in order to address the question of the effects of reproductive tissue as a strong sink for the carbohydrates produced in the fronds, as well as the physical effects of the sori on one of the photosynthetic surfaces of the fronds.

Material and Methods

Study Site and Plants.—The study site was a subtropical forest at 600 m elevation in the Fushan Experimental Forest in northeastern Taiwan (longitude 121°34′ E, latitude 24°46′ N). Plants were selected in a partially disturbed section of the forest to allow easy accessibility for *in situ* measurements of frond gas exchange. Climatic conditions at the Fushan site are subtropical, with monthly average air temperatures ranging from 10 to 25°C and monthly rainfall ranging from less than 10 cm to over 50 cm, with maxima occurring in the summer months. During the time of measurements for this study (16–23 June 2001), local environmental conditions were: maximum photosynthetic photon flux densities (PPFD) at mid-day in the open of 1300 μmol m^{-2} s^{-1} (although most of the plants used in this study occasionally received this level of PPFD, shading by the host tree and neighboring trees more often reduced the PPFD the plants received), average air temperature of 23.4°C, average air relative humidities (RH) over 80%, and average daily wind speeds from 1.3 to 3.5 m s^{-1}. Most days of the study were intermittently cloudy without precipitation.

Large, sprawling individuals of *Pyrrosia lingua* (Thunb.) Farw. were growing epiphytically on host trees that included *Litsea acuminata* (Bl.) Kurata (Lauraceae), *Machilus zuihoensis* Hayata (Lauraceae), *Castanopsis cuspidata* (Thunb. *ex* Murray) Schottky var. *carlesii* (Hemsl.) Yamazaki (Fagaceae), *Pasania hancei* (Benth.) Schottky (Fagaceae), *Engelhardia roxburghiana* Wall. (Juglandaceae), and *Lagerstroemia subcostata* Koehne (Lythraceae); vouchers of the fern were deposited in the herbarium of the Taiwan Forestry Research Institute, Taipei (TAIF). Epiphytes and hosts were chosen for study primarily for two reasons: easy access from the ground or a ladder and abundance of sterile and fertile fronds. All plants selected were growing several meters along the length of the main stem of the host trees. Different fronds were used for each of the physiological measurements, and another frond was used for all morphological measurements. In all cases, the sample size was six fronds, each from a different plant.

FROND MORPHOLOGY.—Frond thickness was measured with an ocular and stage micrometer and a Leica (Mannheim, Germany) DMLB microscope. Stomatal density and stomatal dimensions were measured on a computer using digitized photomicrographs taken with this microscope using the middle section of a freshly cut mature frond of average length for each plant. Stomatal measurements were made using fingernail polish impressions of the frond surfaces. Ten stomata were measured on each frond; mean dimensions were used for each of the six plants examined. For the fertile fronds, stomata were measured after removal of the sori.

FROND OSMOTIC POTENTIAL.—Within 15 minutes of detachment, four 1.2-cm diameter disks were punched from the middle of a mature frond of average length and frozen at −10°C. After at least 24 hours, the disks were thawed, then pressed in a vice until a filter paper disk was saturated with the expressed liquid. The osmotic potential of this liquid was then measured with a Wescor (Logan, UT) Model 5500 Vapor Pressure Osmometer, using standards of known osmotic potentials for calibration.

FROND CHLOROPHYLL CONCENTRATION.—Within 15 minutes of detachment, four 1.2-cm diameter disks were punched from the middle of a mature frond of average length and placed in 20 ml of *N,N*-dimethylformamide (DMF). After two days in the dark at room temperature, the disks were colorless. The chlorophyll (*a* and *b*) concentrations of the DMF solution was measured with a Hach (Loveland, CO) Model DR/3000 spectrophotometer according to Moran (1982). Absorbances at 720 nm were negligible, indicating that the extracts contained few contaminants. The disks were recovered and dried at 70°C for a minimum of one week before weighing.

FROND WATER CONTENT DURING DESICCATION.—The bases of fronds were cut underwater, and the cut end kept underwater during transport to the laboratory (about 15 minutes), after which the fronds were placed under fluorescent lamps for 30 minutes (cut end still immersed). Then the distal 10–15 cm section of the fronds was excised, weighed, then laid, adaxial side up (supported by an empty styrofoam cup wider than the frond segment), on a lab bench under the same lamps. The fronds were then weighed every five minutes for 1.5 hours. Environmental conditions at frond level were 60–100 μmol m^{-2} s^{-1} PPFD, 29–30°C, approximately 70% RH, and 380–420 ppm CO_2. After the desiccation period, the fronds were placed in an oven at 70°C for at least a week before weighing.

IN SITU FROND GAS EXCHANGE.—Gas (CO_2 and H_2O vapor) exchange of a 2 × 3 cm area in the middle of a mature frond of average length was measured *in situ* using a LI-COR (Lincoln, NE) LI-6400 Portable Photosynthesis System under the following controlled conditions: 500 μmol m^{-2} s^{-1} PPFD (red and blue diodes) on the adaxial surface, 27–30°C leaf temperature, 70–80% RH, and 360–380 ppm CO_2 concentration. Several prior photosynthesis-PPFD curves using sterile fronds indicated that 500 μmol m^{-2} s^{-1} PPFD was a near-saturating light level. Environmental conditions outside the gas exchange

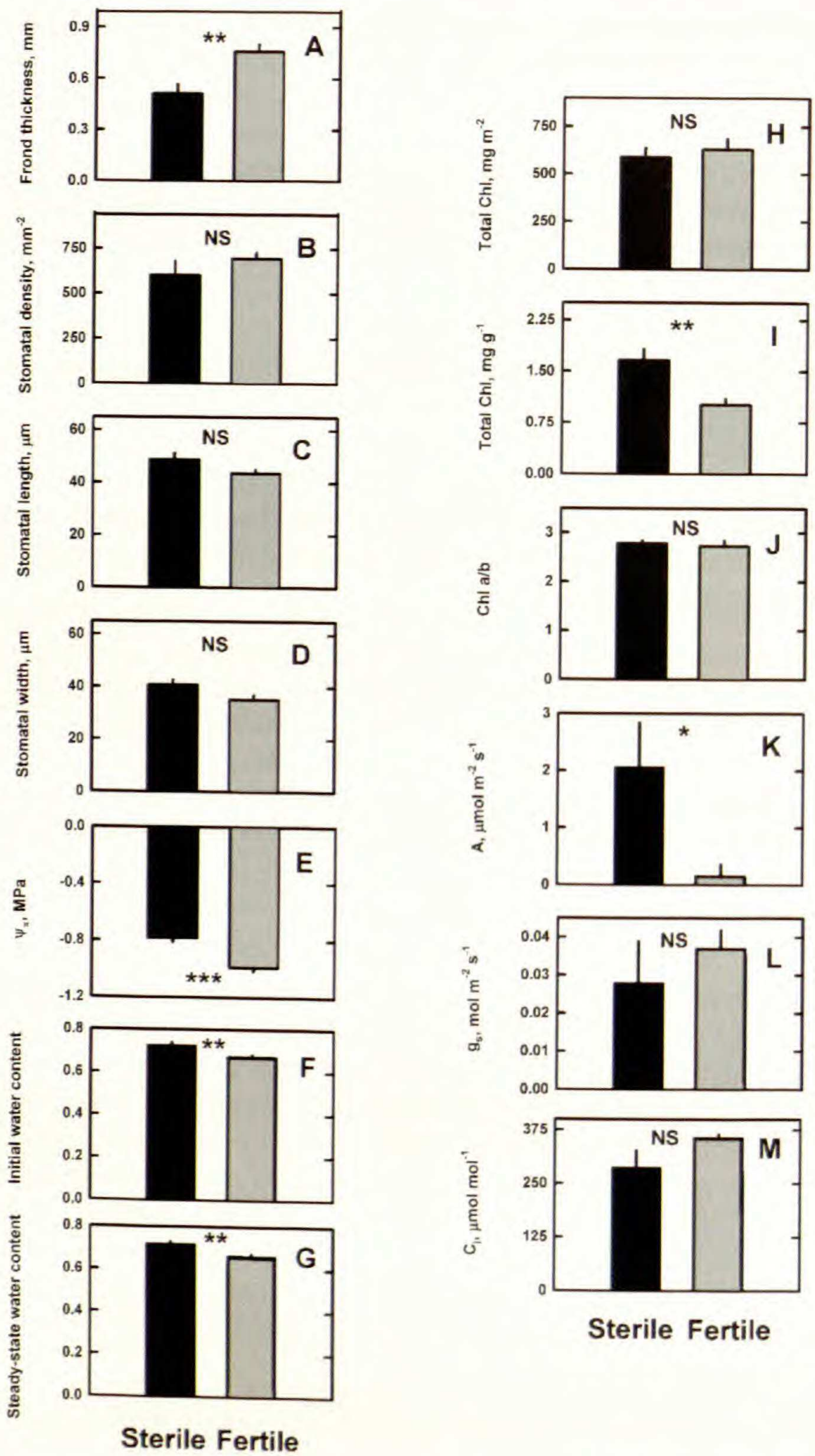

FIG. 2. Mean (bars indicate standard errors; N = 6 plants) frond thicknesses (A), stomatal densities (B), stomatal lengths (C), stomatal widths (D), osmotic potentials (E), initial water contents at the beginning of a desiccation experiment (F), steady-state water content later in the desiccation experiment when water loss rates stabilized (G), chlorophyll concentrations on an area basis (H), chlorophyll concentrations on a fresh mass basis (I), chlorophyll *a/b* ratios (J), *in situ* rates of net CO_2 exchange (K), *in situ* stomatal conductances (L), and *in situ* internal CO_2 concentrations (M) of

chamber varied from plant to plant, primarily a result of shading by surrounding vegetation. Data were collected only when gas exchange reached a steady-state level, which typically took fifteen minutes.

STATISTICAL ANALYSIS.—Means of the morphological and physiological data for sterile and fertile fronds were compared using the Student's *t*-test or, when comparisons did not meet the assumptions of the *t*-test, a Mann-Whitney *U*-test was applied (Sokal and Rohlf, 1981). Frond thicknesses were compared with a one-way analysis of variance, followed by a Tukey test to compare differences of individual means. Statistical significance was inferred when $P \leq 0.05$. Statistical analyses were performed using the software program SigmaStat (SPSS Inc., Chicago).

RESULTS AND DISCUSSION

Most species of *Pyrrosia* do not exhibit sterile/fertile frond dimorphism (Hovenkamp 1986), although subtle differences between shape and/or size are nearly always found in such "monomorphic" taxa (Wagner and Wagner, 1977; also see Fig. 1). Hovenkamp (1986) claims that sterile fronds are found only in young plants or plants growing under unfavorable conditions, and that, once mature, all fronds subsequently produced will harbor sori if environmental conditions permit. The latter claim does not fit observations of *P. lingua* in the subtropical forest in Taiwan. Specifically, although detailed phenological data are lacking, many fronds remain sterile for years and apparently for their entire lives. In the "*P. lingua* group" sterile fronds are only slightly wider and shorter than the fertile fronds, and this difference is minimal in *P. lingua* (Hovenkamp 1986; also see Fig. 1).

Although the coverage of sori on the abaxial surfaces of fertile fronds of the epiphytic fern *Pyrrosia lingua* varied from approximately 25% to 100% of the entire frond (personal observation), only fronds with nearly complete coverage were included in this investigation (Fig. 1). Such fertile fronds were substantially thicker than sterile fronds (Fig. 2A). Based on measurements of the thickness of fronds in the narrow spaces between the sori, the difference in thickness was clearly attributable to the large sori on the abaxial surfaces of the fertile fronds. Stomatal widths, lengths, and densities were not different between the two types of frond in this fern (Fig. 2B–D).

The osmotic potential of the liquid expressed from the fertile fronds was more negative than the liquid from the sterile fronds (Fig. 2E). It is unclear

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sterile and fertile fronds of *Pyrrosia lingua* in northeastern Taiwan. *** signifies a difference in the means at $P \leq 0.001$; ** signifies a difference in the means at $P \leq 0.01$; * signifies a difference in the means at $P \leq 0.05$; NS signifies no difference ($P > 0.05$). In figure A, mean frond thickness between sori was 0.60 mm (standard deviation = 0.03), which was not significantly different from the thickness of the sterile fronds ($P > 0.05$). In figures F and G, frond water content = [(fresh weight) − (dryweight)]/(fresh weight).

whether this reflects a real difference in osmotic potential between the two types of fronds or an artifact due to a matric effect of the spores released during extraction of the frond liquid.

Early in the frond desiccation experiment, sterile fronds contained more water than the fertile fronds (Fig. 2F), which is the opposite of expectations if the difference in osmotic potential between the two frond types was real. In addition, the sterile fronds maintained a greater hydration level once steady-state rates of water loss were reached later in the desiccation period (Fig. 2G). Rates of water loss of both types of fronds were similar (data not shown).

Although chlorophyll concentrations on a mass basis were greater in the sterile fronds than in the fertile fronds (Fig. 2I), there were no differences between the two frond types when concentrations were expressed on an area basis (Fig. 2H). A likely explanation for this discrepancy is that the additional mass of the non-chlorophyllous sporangia and spores reduced the chlorophyll concentration per mass in the fertile fronds. Chlorophyll *a/b* ratios did not vary between the different fronds (Fig. 2J).

In situ rates of net CO_2 uptake of the sterile fronds of *P. lingua* were very low and highly variable, a finding not unlike those found in other studies of the ecophysiology of epiphytic ferns (Kluge *et al.*, 1989; Stuntz and Zotz, 2001). Furthermore, rates of CO_2 uptake in the fertile fronds were significantly and substantially lower than those of the sterile fronds (Fig. 2K). The presence of sori on the abaxial surfaces of the fertile fronds may have prevented complete stomatal opening, or they may have physically blocked the diffusion of CO_2 into the fronds. This seems unlikely given the observed lack of differences in stomatal conductance between the two frond types (Fig. 2L). The gas exchange data, in particular the lower CO_2 uptake rates of the fertile fronds, coupled with similar internal CO_2 concentrations (Fig. 2M) and conductances, provide some indication that the lower net CO_2 uptake rates of the fertile fronds are due, in part, to a reduction in photosynthetic capacity. This could be the result of shading of the photosynthetic tissue by the sori obscuring the abaxial surface of the vertically oriented fertile fronds. On the other hand, the observed lack of differences in chlorophyll concentrations and *a/b* ratios between the two types of fronds (Fig. 2H,J) does not support this speculation, as shade-acclimated leaf tissue typically has a higher chlorophyll concentration and a lower chlorophyll *a/b* ratio than does sun-acclimated tissue (Boardman, 1977; Björkman, 1981). Furthermore, in all photosynthetic measurements in this study, light impinged only on the adaxial surfaces of the fronds.

It is possible that the sori did indeed physically impede gas exchange in the fertile fronds, yet the calculated "stomatal" conductances were elevated as a result of water loss directly by the sori. In this case, CO_2 uptake rates of the fertile fronds would be lower than those of sterile fronds, yet frond conductances would remain the same, precisely as observed in this study. Unfortunately, little is known about the gas exchange features of the sori. Clearly, further work is necessary to determine the precise causes of the decreased photosynthetic capacity in the fertile fronds. These results of greatly reduced photosynthetic rates in fertile fronds, relative to sterile fronds, in

Pyrossia lingua are in direct contrast to the results obtained with *Dryopteris filix-mas* (Bauer *et al.*, 1991). The difference between these two taxa might reflect the difference in sori coverage of the abaxial surfaces of the fronds, as the sori obscure much less of the frond surface in *D. filix-mas* (Page, 1982), relative to *P. lingua*.

In summary, the fertile fronds of the subtropical epiphytic fern *Pyrrosia lingua* are thicker, have more negative osmotic potentials, lose water more easily, and have much lower photosynthetic rates than sterile fronds. Although many of these differences may be ascribed to the physical (morphological) nature of the sori on the fertile fronds, some evidence was found for differences in the photosynthetic capacity of the fertile versus the sterile fronds. The results of this study clearly indicate that reproduction in this fern is accompanied by a physiological cost in the form of reduced photosynthetic rates for the fertile fronds. Given the large number of fertile fronds often found on individual plants, this cost could prove substantial to reproductive individuals of this fern.

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